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Chitosan-oxychitin coatings for prosthetic materials

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Abstract

Plates of Ti-6Al-4V alloy were plasma-sprayed with hydroxyapatite and with bioactive glass. Chitosan acetate solution was then used to deposit a chitosan film upon the plasma-sprayed layers, which was further reacted with 6-oxychitin to form a polyelectrolyte complex. The latter was optionally contacted with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide at 4°C for 2 h to form amide bonds between the two polysaccharides. Uniform flat surfaces exempt from fractures were observed with an electron microscope. The chitosan film was also submitted to acetylation with acetic anhydride in methanol to obtain a chitin film. In all cases the modified chitosan films were insoluble. The results are useful for the preparation of prosthetic articles possessing an external organic coating capable to promote colonization by cells, osteogenesis and osteointegration. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Chitosan; Oxychitin; Prosthetic materials; Bioglass; Bioceramics; Hydroxyapatite

1. Introduction

Metallic implants such as Ti and its alloys are currently coated with plasma-sprayed hydroxyapatite. It is believed that bioactive ceramics bond to bone through a layer of bone-like carbonate-containing hydroxyapatite, which is formed on their surface in vivo. The ceramic layer favours the integration between newly formed bone tissue and prosthesis, nevertheless it appears hardly to be accessible to bone cells. To enhance the integration of the implant and to promote the regeneration of bone, chitosan has been used in association with various forms of calcium phosphate. For example, the dicarboxymethylchitosan-calcium phosphate complex has been used in vivo to repair surgical lesions (Muzzarelli et al., 1998); tooth-derived hydroxyapatite in admixture with chitosan has been used in dental surgery (Pal, Pal, Mukherjee & Pal, 1997); and a chitosan-bonded bone filling paste was developed (Ito & Idaka, 1997; Kawakami, Antoh, Hasegawa, Yamagishi, Ito & Eda, 1992; Maruyama & Ito, 1996).

The rationale behind the use of chitosan for this purpose is that chitosan has osteoconductive properties and is highly biocompatible and resorbable (Muzzarelli, Biagini, Bellardini, Simonelli, Castaldini & Fratto, 1993a; Muzzarelli et al., 1993b; Muzzarelli et al., 1997); chitosan also promotes nucleation and growth of apatite and calcite crystals as well (Kato & Amamiya, 1999).

Besides these appealing characteristics, chitosan shows polycationic behaviour and therefore forms polyelectrolyte complexes with polyanions (Kubota & Kikuchi, 1998); it is also a highly filmogenic biomaterial (Muzzarelli, Isolati & Ferrero, 1974).

Consecutive deposition of oppositely charged polyelectrolytes from dilute 0.5 M NaCl onto charged solid supports was introduced recently (Decher, 1997), and polymeric shells were accordingly manufactured (Donath, Sukhorukov, Caruso, Davis & Mohwald, 1998) and described as extremely stable against chemical and physical influences, notwithstanding their small thickness, typically 20 nm for a nine-layer shell. Improved alternate deposition of chitosan and poly(γ-glutamic acid) on quartz was reported (Serizawa, Goto, Kishida, Endo & Akashi, 1999). It thus appears that polyelectrolyte complex formation would be of interest in order to coat a prosthetic article in a permanent way and to make the chitosan coating more resistant during implantation, when, for a short while, it is submitted to mechanical stress.

Taking advantage of previous experience with chitosan-coated hydroxyapatite (Mattioli-Belmonte et al., 1995; Muzzarelli, Mattioli-Belmonte, Pugnaloni & Biagini, 1999a), the present work was undertaken with the intention of verifying the possibility of coating with chitosan the plasma-sprayed hydroxyapatite and glass layers deposited on orthopedic alloys, and to modify chemically the

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Table 1
Nominal compositions of the hydroxyapatite and the AP40 bioactive glass

Compound	(wt%)
Hydroxyapatite powder	
Hydroxyapatite, $Ca_5(PO_4)_3(OH)$	≥ 98.0
Calcium oxide, CaO	≤ 0.5
α-Tricalcium phosphate, $Ca_3(PO_4)_2$	≤ 0.5
β-Tricalcium phosphate, $Ca_3(PO_4)_2$	≤ 0.5
TTCP, $Ca_4(PO_4)_2O$,	≤ 0.5
Ca/P ratio 1.660 ± 1.681	
Crystallinity index ≥90%	
AP40 Bioactive glass powder	
SiO_2	44.00
CaO	32.16
MgO	2.84
CaF_2	5.00
Na_2O	4.60
K_2O	0.20
P_2O_5	11.20

deposited chitosan film in order to depress chitosan solubility and swelling, and stabilise the organic layer. 6-Oxychitin [(1 → 4)-2-acetamido-2-deoxy-β-D-glucuronan], a regiospecifically carboxylated chitin that we have tested in bone regeneration (Mattioli-Belmonte et al., 1999; Muzzarelli, Muzzarelli, Cosani & Terbojevich, 1999b) and in the preparation of microcapsules for drug delivery (Muzzarelli et al., 2000) is very effective in the formation of polyelectrolyte complexes with chitosan: thus a polyelectrolyte complex can be prepared from a polyamine (chitosan) and a polycarboxylate (6-oxychitin) both derived from the same chitin. This means that the coating would be endowed with the highest biological significance.

2. Experimental

2.1. Chemicals

All chemicals were analytical reagent grade and were supplied by Aldrich, Milano. Tyrosinase from mushroom (Sigma T7755) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide were supplied by Sigma, Milano.

2.2. Chitins

Shrimp chitin was supplied by Primex, Norway. 6-Oxychitin was obtained as described elsewhere (Muzzarelli et al., 1999b) with the aid of Tempo™ as a regiospecific catalyst of the oxidation of the primary alcohol group to a carboxylate group. It was used as 6-oxychitin sodium salt aqueous solution, average MW 9000 Da, degree of carboxylation 1.0. Chitosan from the same chitin had degree of acetylation 0.80 and average MW ca. 200 kDa, and was used as chitosan acetate 1% aqueous solution.

2.3. Glass and hydroxyapatite coatings

Plates of Ti-6Al-4V alloy (10 × 10 × 0.5 mm) were sand-blasted and used as supports for plasma-spray deposition of hydroxyapatite and AP40 bioactive glass. The hydroxyapatite powder was supplied by Flametal SpA, Fornovo di Taro (Parma), Italy; the bioactive glass powder was supplied by CNR-IRTEC, Faenza, Italy (Krajewski, Malavolti & Piancastelli, 1996). Their nominal compositions are in Table 1. Both materials were characterised by x-ray diffraction spectrometry (XRD), scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS), before and after deposition (powders and coatings, respectively). The thickness of the deposited layers was determined by SEM, after inclusion in epoxy resin and cutting with the aid of a diamond saw. The cross-section was prepared with grinding papers and polished with diamond paste. The samples were graphite-coated before SEM and EDS. SEM observations and local EDS analyses were performed on the cross-section, in order to evaluate the composition variations through the thickness of the coating.

2.4. Instruments

The FTIR spectra were obtained with the Perkin–Elmer Spectrum 2000 FTIR spectrometer equipped with a i-series microscope mounting a micro-ATR objective, resolution 4 cm^{-1}, 250 scans. Of each sample, three different micro-sites of the surface were analysed: thus the spectra can be considered an average of the spectral results. To validate the microanalysis findings, a small zone of the surface of each specimen was scraped with a lancet, and the resulting powder dispersed in KBr was analysed with a Nicolet 20-SX FTIR spectrometer equipped with a Spectra Tech "Collector" accessory for DRIFT measurements. A very close spectral similarity could be evidenced between these spectra and those from Micro-ATR.

XRD investigations were performed with an INEL diffractometer equipped with a curved position sensitive detector CPS 120 and a Bruker D8 Advance diffractometer in thin film configuration using Cu-Kα radiation for both instruments. SEM observations were carried out with a Philips XL20 electron microscope equipped with an EDAX PV 9800 microanalysis system.

3. Results and discussion

3.1. Hydroxyapatite

The hydroxyapatite powder was a mixture of crystalline hydroxyapatite [$Ca_5(PO_4)_3(OH)$, ICDD card 9-432], calcium phosphate [$Ca_3(PO_4)_2$ β-TCP, ICDD card 9-169] and calcium oxide [CaO, ICDD card 28-775] based on XRD analysis. SEM observations showed spherical particles 3–60 μm in diameter.

After plasma spray deposition the coated plates exhibited

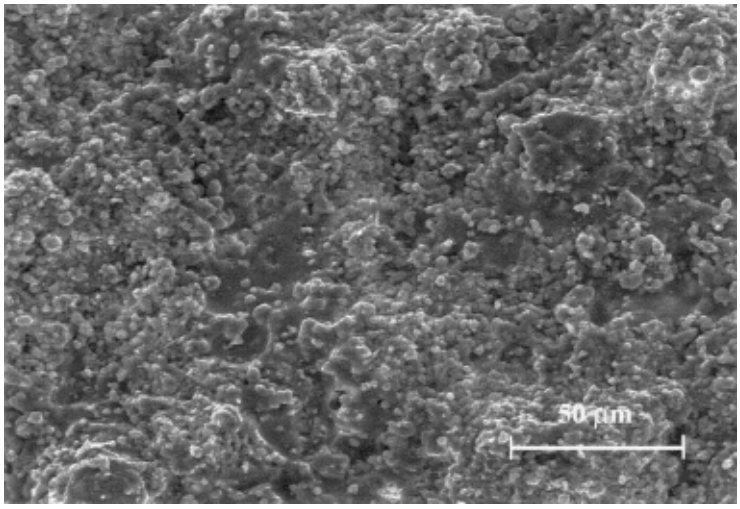

Fig. 1. Scanning electron micrographs of the surface of the plasma sprayed hydroxyapatite coating.

very rough and porous surface with the presence of melted zones (Fig. 1); inside the melted zones small cracks were visible at higher magnifications. The microanalysis showed only Ca and P with a ratio depending on the value of the voltage used in the SEM. In particular, at 30 kV the atomic Ca/P ratio was 2.03 whereas it was reduced to 1.63 at 10 kV, which is very close to the nominal ratio (1.67) of the stoichiometric hydroxyapatite. This suggests an increase of the Ca content with depth.

The thickness of the hydroxyapatite coating was 50–80 μm. In cross-section, the layer appeared very irregular due to compact melted zones alternating with very porous ones, where the single particles of hydroxyapatite were still visible (Fig. 2). The interface with the support was continuous with no segregations. In Fig. 3, x-ray spectra of the hydroxyapatite powder are compared with the spectra of the hydroxyapatite coating on the Ti support, recorded at incidence angles 0.2, 2 and 5° in thin film configuration. From the XRD spectra, it is inferred that the deposited layer has the same crystal structure as the powder, but reduced crystallinity, and both β-TCP and CaO are preferentially located deep in the layer. The presence of CaO justifies the increased Ca/P ratio in the depth of the layer as obtained by EDS. In conclusion, the layer is made of stoichiometric hydroxyapatite with an increase of calcium oxide with depth. Information on the presence of β-TCP can be exclusively obtained by XRD, since the values of the Ca/P ratio for β-TCP and hydroxyapatite are very close together and in the range of the experimental errors of the EDS technique.

3.2. Bioactive glass

The powder contained irregularly shaped crystals (2–50 μm). The XRD spectra evidenced that the powder was rather crystalline and the diffraction peaks were attributed to

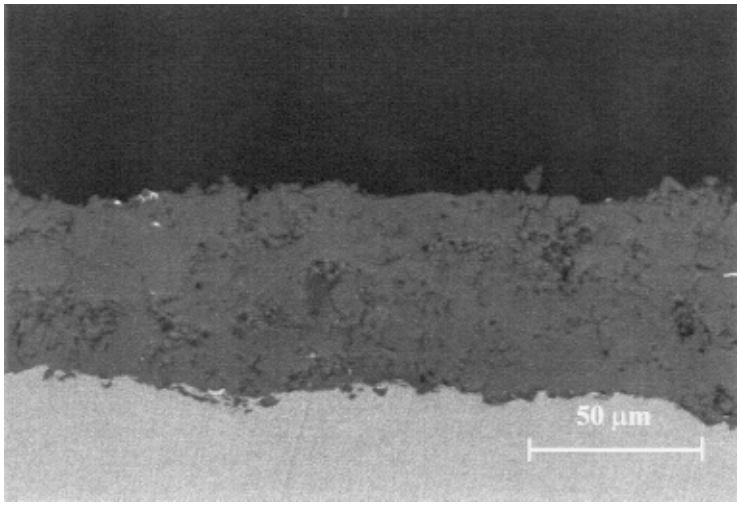

Fig. 2. Scanning electron micrograph of the cross-section of the hydroxyapatite coating.

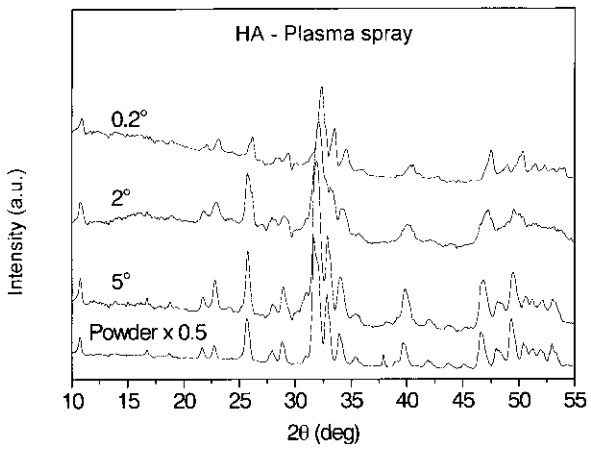

Fig. 3. X-ray diffraction patterns of the hydroxyapatite powder (half intensity) compared with the patterns of the hydroxyapatite coating taken at three incidence angles (0.2, 2 and 5°).

hydroxyapatite (ICDD card 9-432) and calcium oxide (ICDD card 28-775). No other elements were detected.

The layer deposited by the plasma spray had an irregular surface characterised by particles with dimensions 1–20 μm (Fig. 4). Most of the particles were melted together, and often very large melted zones (>50 μm diam.) were observed. The cracks inside the larger melted zones were less numerous and thinner than the hydroxyapatite layers. The thickness of the coating was 5–30 μm. In cross-section (Fig. 5) the layer had a sharp boundary and few holes dispersed inside it. The spectra of the AP40 powder together with the spectra of the coating taken at different incidence angles are in Fig. 6. The crystal structures of the deposited layer and the powder were similar, but the total crystallinity was lower and the elemental microanalysis did not show any appreciable variation with respect to the powder.

The aspect of both types of inorganic coatings indicated their suitability for holding a chitosan film. It is known that chitosan strongly adheres to glasses and ceramics when films are cast from chitosan solutions. A more marked adhesion to hydroxyapatite and bioglass was therefore expected in consideration of the rough surfaces. Many particles that were in the upper part of the layer actually protruded from the upper surface, thus anchoring the chitosan films more firmly.

3.3. Preparation of the organic coatings

3.3.1. Chitosan coating

A deaerated chitosan acetate solution (1 g chitosan in 100 ml of 1% acetic acid) was poured in a Petri dish containing the hydroxyapatite-coated alloy plates (coating upward) in such a quantity as necessary to submerge the plates under the desired thickness of solution (3 mm). The film was formed by drying in an oven at 60°C overnight. Immediate macroscopic evidence of chitosan was obtained by reaction with 3,4-dihydroxybenzaldehyde in water (yielding a yellow color) and with OsO_4 (dark brown color) (Pearlmutter & Lembi, 1978). These coated plates were used for the following chitosan reactions. The FTIR spectrum of the chitosan acetate-coated plates, in Fig. 7, showed the expected characteristic bands of this compound at 1662 and 1592 cm^{-1}.

3.3.2. Chitosan-oxychitin coating

A solution of oxychitin (0.2 g in 10 ml water) was contacted with chitosan acetate-coated plates under very mild shaking for 10 min. The plates were dried in a desiccation vessel. At the SEM observation, the organic coating was quite uniform, no fracture being visible.

The chitosan acetate + oxychitin coated plates were further treated with water-soluble 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (0.2 g in 4 ml water) for 2 h at 4°C, in order to promote amide bond formation between the

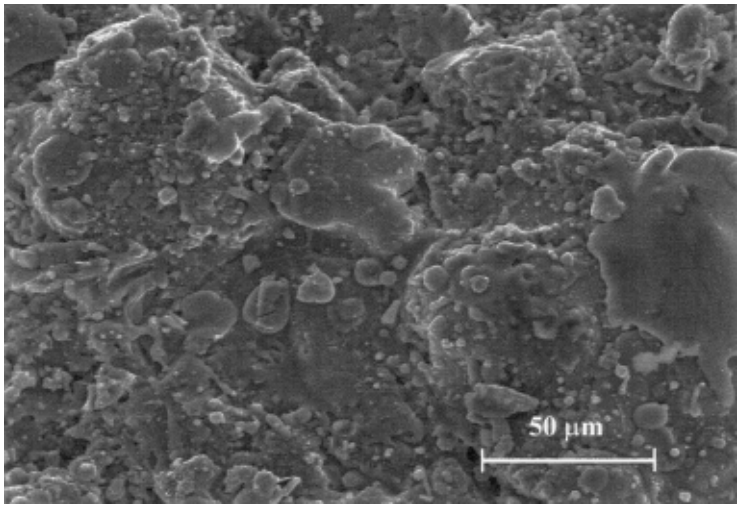

Fig. 4. Scanning electron micrographs of the surface of the plasma sprayed AP40 glass coating.

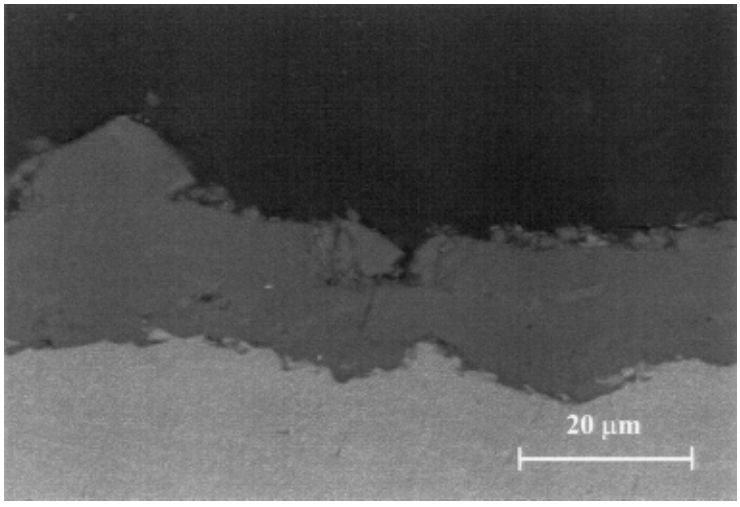

Fig. 5. Scanning electron micrograph of the cross-section of the AP40 coating.

two polysaccharides. This preparation, observed at 400×, appeared quite flat and uniform with no fractures.

The vibrational modes of oxychitin (amide I as well as H-bonded NH bending) gave rise to the typical composite band with a maximum at 1612 cm^{-1} (Fig. 7), indicative of the presence of ionised carboxylate groups. After the 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide treatment, the FTIR spectrum showed the intermolecular amide linkage of chitosan to oxychitin at 1630 cm^{-1}. This most remarkable addition in the amide region was an indication of the amide bond formation between chitosan and oxychitin (Fig. 7).

3.3.3. Chitin coating

The chitosan acetate-coated plates were submitted to acetylation according to the protocol, leading to a degree of acetylation close to 1.0 (East & Qin, 1993; Hirano, Yoshida & Takabuchi, 1993) with the aid of acetic anhydride in methanol (500 mg in 20 ml). The solution in contact with the plate was continuously stirred overnight and was washed repeatedly with methanol. A smooth and regular surface was evidenced by SEM; the relevant FTIR spectrum showed the presence of the typical chitin bands at 1679, 1577, 1438, 1388, 1326, 1170, 1132 and 1093 cm^{-1}.

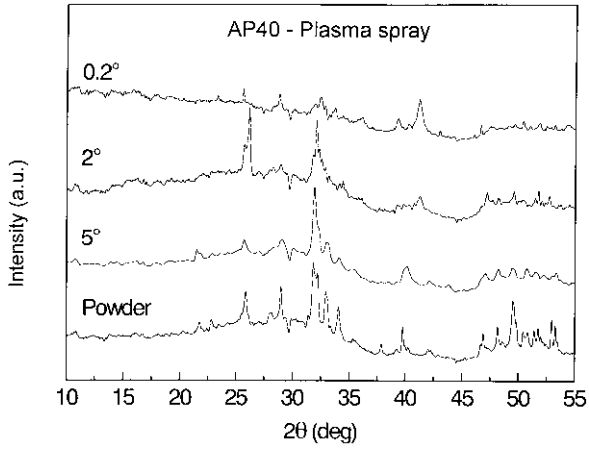

Fig. 6. X-ray diffraction patterns of the AP40 powder compared with the patterns of the AP40 coating taken at three different incidence angle (0.2, 2 and 5°).

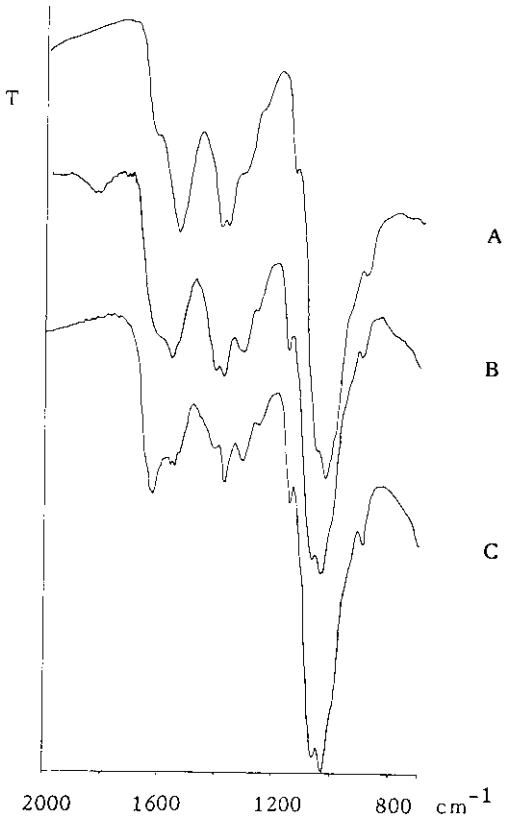

Fig. 7. Infrared spectra recorded on the treated hydroxyapatite surfaces: A) chitosan coated; B) chitosan and 6-oxychitin coated; C) chitosan and 6-oxychitin coated in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide.

3.3.4. Other coatings

Chitosan coatings treated with 3,4-dihydroxybenzaldehyde and tyrosinase (Muzzarelli, Ilari, Xia, Pinotti & Tomasetti, 1994) were unsatisfactory due to the presence of imperfections and scattered aggregations of organic material, possibly from the aldehyde treatment.

4. Conclusions

The experimental results indicate that chitosan acetate can be easily used to cast a film of chitosan on plasma-sprayed hydroxyapatite. The upper surface of the chitosan films is smooth, while the lower surface matches the irregularly shaped contours of the hydroxyapatite particles. While there are indications in the literature that chitosan acetate can be thermally converted into chitin (Lim, Khor & Ling, 1999; Lim & Wam, 1995; Toffey, Samaranayake, Frazier & Glasser, 1996), heating at temperatures higher than 100°C was considered unsatisfactory for the present investigation. The inherent solubility of chitosan acetate that would presumably lead to damage and loss of the chitosan coating during surgery, could be depressed more conveniently by either polyelectrolyte complex formation with 6-oxychitin sodium salt, or optional further treatment with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, or acetylation in methanol. In all cases the coating was uniformly flat and smooth. The polysaccharide components were clearly identifiable by FTIR.

Prosthetic articles coated with these polysaccharides capable to promote colonization by cells, osteogenesis and osteointegration could therefore be prepared. In the light of recent data on chitosan-hydroxyapatite associations for bone implants (Ito, Ikada, Nakajima, Yagasaki & Kafrawy, 1999; Tamahashi, Kobuko, Naskamura, Katsura & Nagano, 1996) it is expected that they are biologically well tolerated.

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